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Effects of Folic Acid Supplementation on Anxiety- and Depression-like Behaviours, and Locomotion in Captive male African Giant Rats (*Cricetomys gambianus*, Waterhouse – 1840) Subjected to Chronic Physical Immobilisation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to evaluate the impact of physical immobilisation and folic acid (FA) supplementation on neurobehaviour in African Giant rats, AGRs (*Cricetomys gambianus*, Waterhouse-1840). Twenty wild adult male captive AGRs were randomly divided into four groups of five rats each. Group 1 AGRs were neither immobilised nor administered FA. Groups 2 and 3 AGRs were subjected to physical immobilisation, which lasted six hours per day for 21 days. Group 3 AGRs, in addition, received FA-supplemented feed, at the concentration of 3.5 mg/kg feed for 10 consecutive days immediately following the immobilisation protocol. Group 4 AGRs were not immobilised, but received FA-supplemented feed as in group 3 AGRs. All AGRs were evaluated for anxiety- and depression-like behaviours, and locomotor activity using Elevated Plus Maze and Open Field Test, respectively. The results showed that both the percentage open arm entry and number of squares crossed were significantly ($P < 0.05$) reduced in the immobilised AGRs (groups 2 and 3), but the reduction was less ($P < 0.05$) in those that received FA post immobilisation (group 3). The percentage open arm exploration was significantly ($P < 0.05$) lower, while the rearing activity was significantly ($P < 0.05$) higher in the immobilised AGRs that received no FA supplementation (group 2). In conclusion, the AGRs that were subjected to chronic physical immobilisation displayed anxiety- and depression-like behaviours, and performed poorly in the tasks for locomotor functions. Post-immobilisation FA supplementation ameliorated the effects.

Keywords: African giant rat; feed supplementation; folic acid; immobilisation; neurobehaviour

1. INTRODUCTION

"Immobilisation is the forced restriction of movement of all or part of an animal's body, either by physical or chemical means. Physical immobilisation methods usually involve traps to restrain the whole animal, or part of the animal or just the use of direct handling restraint" [1]. "Immobilisation of an undomesticated or anxious animal may cause considerable stress" [2]. "When animals are immobilised they may undergo some or all of a series of acute stressors, including pursuit, restraint, pain, fear and anxiety, all of which are capable of inducing harmful responses" [2,3,1]. Chronic physical immobilisation (CPI) may be associated with impairments in working memory, spatial learning, neurogenesis, enhanced anxiety-like behaviour, depression and reduced long-term potentiation [4-7].

Folate is the naturally occurring form of vitamin B_9, the active form being known as levomefolic acid or 5-methyltetrahydrofolate [8]. "Folic acid (FA) is a synthetic form of vitamin B_9, also known as pteroylmonoglutamic acid. Unlike most folate, the majority of FA is not converted to the active form of vitamin B_9 in the digestive system. Instead, it is converted in the liver or other tissues" [9]. "Studies have shown that there is a correlation between mental symptoms, especially depression and cognitive decline, and high incidence of folate deficiency" [10]. Supplementation with FA in brain dysfunction may improve memory [11].

Most frequently, behavioural changes in animal models are learning and memory loss, anxiety, depression, and motor dysfunction [12,7]. A large variety of rodent behavioural tests are used to evaluate traits such as sensory and motor functions, social interactions, anxiety-like and depression-like behaviour, substance dependence and various forms of cognitive functions [7]. Historically, a large variety of species have been used for behavioural testing [13], but rodents have always been widely used because they are mammals and easy to house and breed. In contrast to common pets, such as cats and dogs, there may also be a higher acceptance in the general public for the use of rodents in medical research. Most behavioural tests used to evaluate sensorimotor functions, learning and memory aim to measure an animal's ability to solve a task [12,13].

The AGR (*Cricetomys gambianus*, Waterhouse-1840) is among the largest muroid rodent species in the world, used largely for meat in Africa [14]. It is a popular and highly priced exotic pet in Europe [15], used for odour detection of landmines and tuberculosis [16,17]. There has been effort to domesticate the AGR [18,19], but its source remains predominantly

from the wild [20-24]. Thus, trap and capture methods are employed before they are kept in cages. These methods may exert tremendous stress on the hitherto free-ranging AGRs, leading to some mortality in captivity. It was, therefore, worthwhile to investigate the effects of CPI on some neurobehavioural parameters in the AGR.

2. MATERIALS AND METHODS

2.1 Location of Experimental Study

The study was carried out in the Department of Veterinary Physiology, Ahmadu Bello University, Samaru-Zaria (11°10′N, 07°38′E), located in the Northern Guinea Savannah zone of Nigeria during the hot-dry season, between March and April, 2019.

2.2 Experimental Animals / Management

African Giant rats were trapped and captured from the wild during the preceding rainy (May – October) and cold-dry (November – February) seasons. Management was carried out as described by Dzenda et al. [20]. Briefly, metal live box traps were baited with fruits, peanuts, maize and ground beans baked cake. The traps were set around suspected AGR burrows and pathways before dusk and checked at dawn. After capture, adult males (bucks) were separated and kept individually in steel cages [15,19], while females (does) and juveniles were released back into the wild. The bucks were housed in a well-ventilated animal room and pre-conditioned for at least two weeks before the commencement of the experiments. They were given access to dry pellet feed and fresh bottled water *ad libitum*. The trap, capture, handling and management methods were performed in conformity with the guidelines of the American Society of Mammalogists [25].

2.3 Experimental Groups and Design

Twenty AGR bucks were divided into four groups of five AGRs each.

Group 1:

The AGRs in this group were not subjected to CPI and remained in their individual spacious cages. They were fed normal feed without FA supplementation.

Group 2:

The AGRs were subjected to CPI in a large metal cage, with partitions measuring 8 cm x 5 cm x 5 cm, for 6 hours per day (between 9:00 – 15:00 h, GMT +1) for 21 days, between 7th and 27th of March, 2019. The sizes of the cage partitions were just large enough to allow an AGR to enter without any extra space to turn. They were fed normal feed without FA supplementation, just like that for group 1 AGRs.

Group 3:

The AGRs were subjected to CPI just as described for group 2 AGRs above. In addition, they were fed with FA (Cika – folic, Enugu, Nigeria) supplemented feed at the concentration of 3.5 mg/kg of feed (Li et al*.,* 2018) for the 10 days that immediately followed the 21 days of immobilisation, between 28th March to 7th April, 2019.

Group 4:

The AGRs were not subjected to CPI, just like group 1 AGRs, but they were fed FA-supplemented feed for 10 days as described for group 3 AGRs above.

2.4 Neurobehavioural and Cognitive Studies

These were carried out a day after the CPI and FA supplementation protocol described above.

2.4.1 Evaluation of anxiety- and depression-like behaviour

This was assessed using the Elevated Plus Maze, EPM [26], measuring 50 cm x 25 cm x 100 cm, modified to accommodate the larger body size of the AGR. It consisted of two open arms (Plate 1) made with plexiglass, in order to prevent the AGRs from escaping, and crossed with two similar closed arms. The arms were connected so that the maze had a look of plus sign. The entire maze was then elevated above the ground level and placed in a quiet and dimly lit room. An AGR was then placed in the centre of the maze facing the closed arms. The following parameters were measured: the number of open arm entries and time spent in the open arms, and similar observations were made for the closed arms. The percentage of

Plate 1. African Giant Rat (*Cricetomys gambianus*) in the Open Arm of Elevated Plus Maze

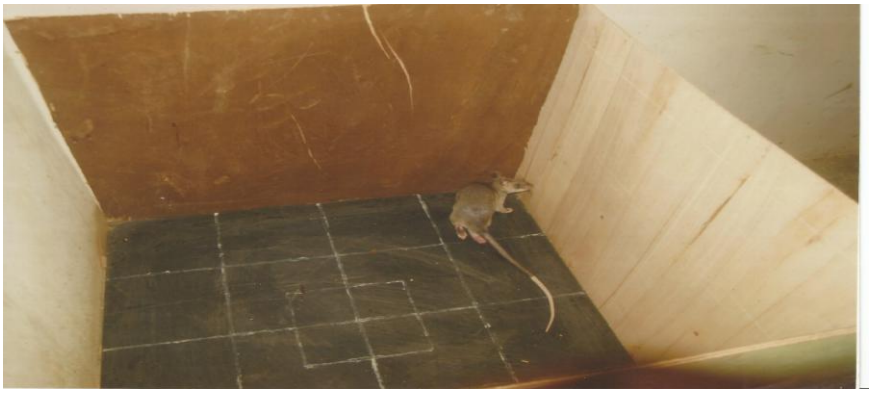

Plate 2. Open Field Apparatus containing African Giant Rat (*Cricetomys gambianus*), showing squares used for measuring locomotor activity

open arm entries were calculated as open arm entries divided by the total number of entries in both open and closed arms. The open-arm exploration, which is time spent in open arm divided by total time spent in both arms, was also calculated.

2.4.2 Evaluation of locomotor activity

This was carried out using the Open Field Test (OFT). This test was also used to measure anxiety and depression-like behaviours in addition to the locomotor activity. The open field apparatus was modified for AGRs, but with the same conformation with the description of Zhu et al. [27] for laboratory rats. The locomotor activity was assessed by placing an AGR in the box and allowing it to roam freely for 3 minutes to familiarise itself with the environment [28]. The number of squares crossed (Plate 2) with all the paws during the next 2 minutes was recorded. The rearing activity was also measured by recording the number of times an animal stood on its hind-limb trying to peep out of the box in the next 2 minutes.

3. RESULTS

3.1 Neurobehavioural Studies

3.1.1 Percentage open arm entry

Results of the percentage open arm entry by AGRs are shown in Fig. 1. The results showed that the mean percentage open arm entry by AGRs was significantly ($P < 0.05$) lower in AGRs of both groups 2 (13.00 ± 1.31%) and 3 (24.62 ± 2.37%) that were subjected to chronic physical immobilisation (CPI), compared to that in groups 1 (40.93 ± 2.05%) and 4 (51.71 ± 4.75%), which were not immobilised. Secondly, of the groups subjected to CPI, the value of the percentage entry recorded for group 2 AGRs was significantly ($P < 0.05$) lower than that obtained for group 3 AGRs, which had received FA supplementation post immobilisation. Thirdly, of the groups of AGRs that were not subjected to CPI, the value of the percentage entry recorded for group 1 AGRs was non-significantly ($P > 0.05$) lower than that obtained for group 4 AGRs, which had received FA supplementation.

3.1.2 Percentage open arm exploration

Results of the percentage open arm exploration by the AGRs are presented in Fig. 2. The percentage open arm exploration was significantly ($P < 0.05$) lower in group 2 AGRs (2.23 ± 2.07%) that were immobilised with no FA supplementation, compared to the values recorded in groups 1 (18.86 ± 1.30%), 3 (10.43 ± 4.73%) and 4 (19.71 ± 6.50%). There was no significant ($P > 0.05$) difference in the percentages obtained between groups 1, 3 and 4 AGRs, although the value for group 3 was relatively lower.

3.1.3 Number of squares crossed

Results of the number of squares crossed within 2 minutes by AGRs in the open field apparatus are presented in Fig. 3. The number of squares crossed by the AGRs was significantly ($P < 0.05$) lower in AGRs of both groups 2 (20.00 ± 1.05) and 3 (26.00 ± 1.90), compared to the numbers in groups 1 (33.20 ± 1.97) and 4 (32.60 ± 1.81), and the value in group 3 was significantly ($P < 0.05$) higher than that in group 2.

3.1.4 Rearing

The numbers of rearing within 2 minutes by AGRs in the Open Field Apparatus are shown in Fig. 4. The rearing activity was significantly ($P < 0.05$) higher in group 2 AGRs (23.40 ± 1.72) than those in groups 1 (11.40 ± 1.29), 3 (12.60 ± 0.81) and 4 (13.40 ± 1.21). The rearing of AGRs was not significantly ($P > 0.05$) different between groups 1, 3 and 4.

3.2 Data Analysis

Data were analysed using one way Analysis of variance (ANOVA) implemented in R car (version 3.0-2) package [29]. Significant differences were separated using Tukey test ($\alpha = 0.05$) for multiple comparisons through R least square means (version 2.30-0).

4. DISCUSSION

The results show that FA supplementation alleviated the effects of chronic physical immobilisation (CPI) on anxiety, depression and locomotion, for the first time, in male AGRs, using the Elevated Plus Maze and Open Field apparatus. The percentages of open arm entry and open arm exploration by AGRs in the EPM were recorded as indices of anxiety and depression, while the numbers of squares crossed and rearing in 2 minutes were obtained as manifestations of motor and sensory functions (locomotor activity).

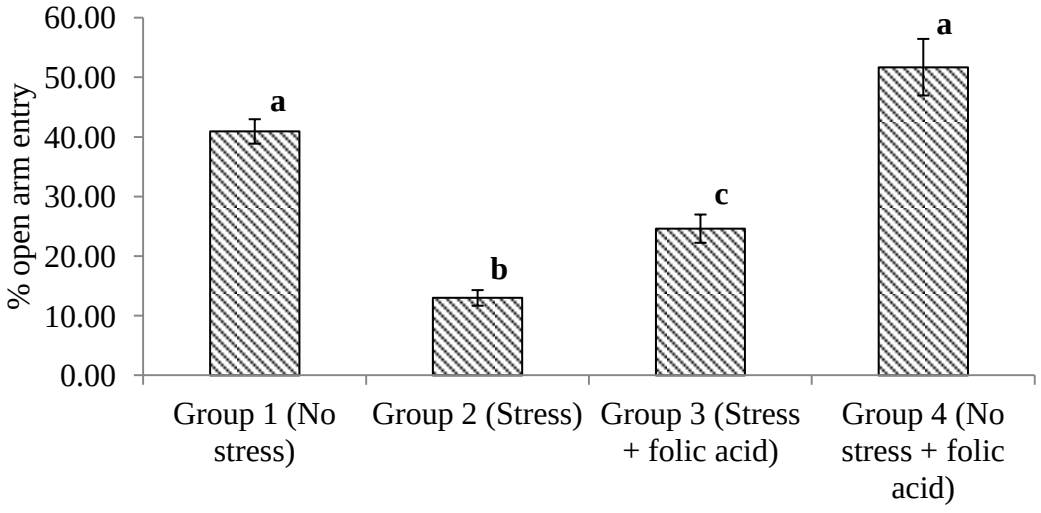

Fig. 1. Effects of chronic physical immobilisation (stress) and folic acid supplementation on Percentage Open Arm Entry of African Giant rats (*Cricetomys gambianus*) in the Elevated Plus Maze. Mean (± SEM) values with different letters (a,b,c) are significantly ($P < 0.05$) different; n = 5 male AGRs per group

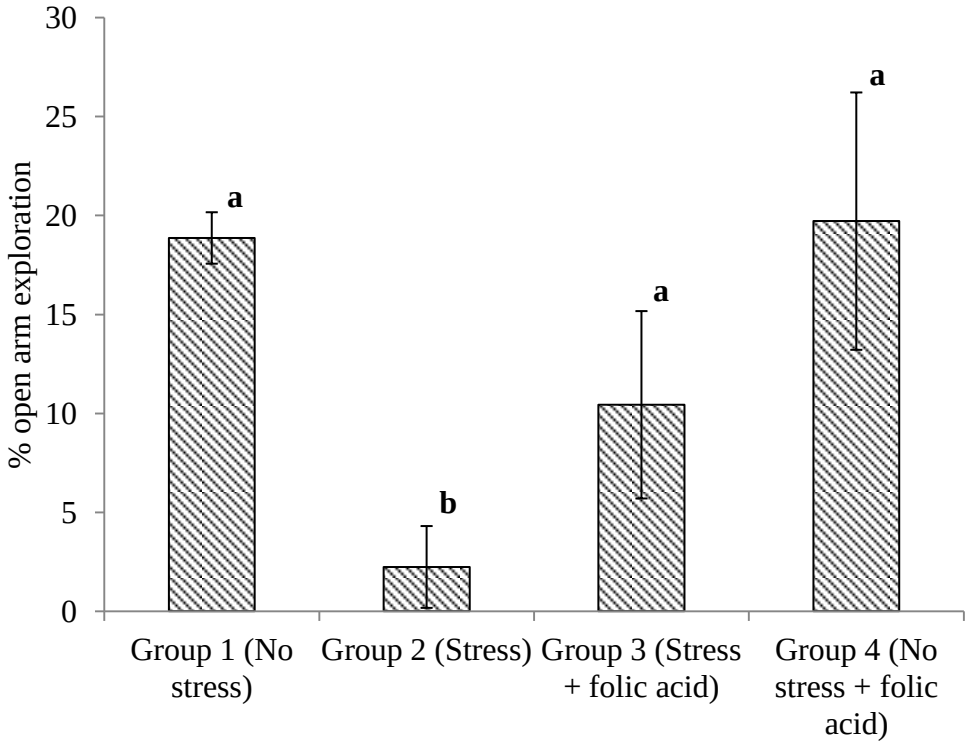

Fig. 2. Effects of chronic physical immobilisation (stress) and folic acid supplementation on Percentage Open Arm Exploration by African Giant rats (*Cricetomys gambianus*) in the Elevated Plus Maze. Mean (± SEM) values with different letters (a,b) are significantly (P < 0.05) different; n = 5 AGRs per group

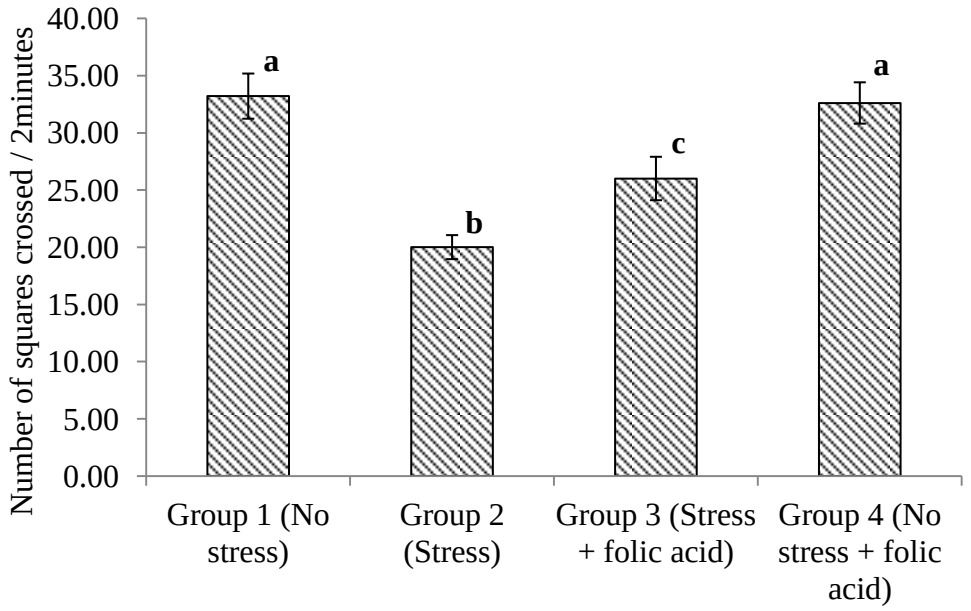

Fig. 3. Effects of chronic physical immobilisation (stress) and folic acid supplementation on the mean number of squares crossed in two minutes by African Giant rats (*Cricetomys gambianus*) in the Open Field Apparatus. Mean (± SEM) values with different letters (a,b,c) are significantly (P < 0.05) different; n = 5 male AGRs per group

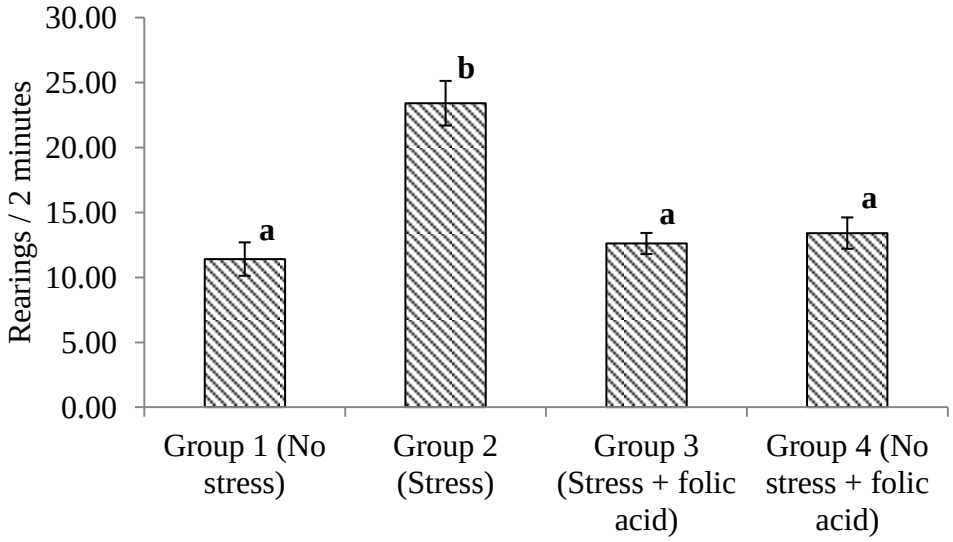

Fig. 4. Effects of chronic physical immobilisation (stress) and folic acid supplementation on the mean number of rearing in two minutes by African Giant Rats (*Cricetomys gambianus*) in the Open Field Apparatus. Mean (± SEM) values with different letters (a,b,c) are significantly (P < 0.05) different; n = 5 per group

The results showed that the percentages of open arm entry and open arm exploration, as well as the number of squares crossed were markedly lower, while the number of rearing was pronouncedly higher in the AGRs that underwent CPI with no FA supplementation after CPI induction. The results demonstrate that CPI-induced anxiety and depression, and affected motor and sensory functions in the AGRs. The findings agree with those of Bhagya et al. [7], Guedri et al. [30] and Jiao et al. [31], who reported that CPI induces neurobehavioural alterations in the laboratory rat as a result of chronic stress-induced impaired hippocampal synaptic plasticity, anxiety and memory deficits. In the present study, there was a significant ($P < 0.05$) decline in the time spent in the open arms and, consequently, a corresponding increase in the time spent in the closed arms by the immobilised AGRs, compared to the control AGRs. The results show a marked increase in anxiety and depression in the immobilised AGRs, which may be due to decreased neurotransmission and neuronal activity [30] caused by the reduction of monoamine neurotransmitters such as 5-hydroxytryptamine and noradrenaline in the central and peripheral nervous system [31]. The results also agree with the works of Qin et al. [32], Chiba et al. [33], Bhagya et al. [7], Guedri et al. [30], Salehi et al. [34], Zain et al. [35] and Jiao et al. [31], but disagree with that of Gregus et al. [36], who reported that repeated restraint stress has no significant effect on anxiety- or depression-like behaviour in male rats. The disagreement could result from differences in the type of behavioural task used as well as species variation. However, Hibicke et al. [37] reported that chronic restraint stress caused depression-, but not anxiety-like behaviour in adolescent female rats.

The results of the current study also show that post-CPI feed supplementation with FA ameliorated the CPI-induced anxiety, depression and sensory-motor dysfunctions in the AGRs, evidenced by the significantly improved percentages of open arm entry and open arm exploration, as well as the number of squares crossed. However, the number of rearing was lowered to levels comparable to those of AGRs that were not subjected to CPI. The finding suggests that feed supplementation with FA was beneficial in accelerating the recovery of AGRs from the neurobehavioural effects of CPI. This may be because FA is an essential vitamin for the development of the central nervous system [38], beneficial in central nervous system functions and development at all ages [39]. Sepehrmanesh et al. [40] obtained similar results while using FA supplementation as augmentation therapy in a major depressive order treatment. The current findings, however,

disagree with those of Lewis et al. [41] in pregnant women, de Koning et al. [42] in older adults, and Okereke et al. [43] in older women, who showed that long-term supplementation with FA had no appreciable effect on anxiety and depression in these groups. The difference in the findings may be due to the fact that all the works were carried out on human subjects, who were not subjected to CPI. The later assertion is, apparently, supported by the current observation that FA supplementation in AGRs that were not previously subjected to CPI did not improve their neurobehavioural indices compared to those of the control AGRs.

The results of the effects of CPI and FA supplementation on basic motor and sensory functions (locomotor activity) evaluated using the open field apparatus show that CPI profoundly affected motor and sensory functions in AGRs by decreasing the number of squares crossed, and increasing the number of rearing by the AGRs. This finding may be due to the effect of immobilisation stress on neuronal activity in several forebrain systems, including limbic structures and the prefrontal cortex [44], compromising normal motor function and accelerating neuronal degeneration [45] and loss [46]. The present findings agree with the works of Metz et al. [47], Smith et al. [45] and Puga et al. [46], that stress modulates motor function in different rat models.

The result showed that post-CPI feed supplementation with FA exerted positive effects on motor and sensory functions in the AGRs since there was marked improvement in the number of squares crossed and complete reversal in the number of rearing to control levels. The results support the finding of Fouda [38] that parenteral FA produces up to 10-fold dose-dependent improvement in *in vivo* regeneration of motor nerves. The FA supplementation could have also suppressed any post-immobilised elevation of plasma total homocysteine [48,49], thus reducing/preventing neuronal vulnerability, and dysfunction [50] in the AGRs. It is also conceivable that the FA reversed CPI-induced reduction by enhancing the propagation of action potentials, leading to improvement in motor and sensory functions [51]. The present findings agree with those of Fouda [38], Shooshtari et al. [50], Kvestad et al. [49], Wang et al. [52,53] and Li et al. [54] that FA improves motor and sensory functions. This study's limitation is that it only includes behavioral experiments; in order to determine the process of how folic acid directly affects nerve transmission, it would be essential to look at biochemical data and other evidence of brain regeneration.

5. CONCLUSION

Chronic physical immobilisation caused anxiety- and depression-like behaviour, and reduced locomotor activity in male AGRs, while post-immobilisation feed supplementation with FA enhanced recovery. More studies with combination of drugs with folic acid should be carried out to justify the novelty of the research.

ETHICAL APPROVAL

Ethical approval for the experiment was obtained from the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria, Nigeria (Number: ABUCAUC/2020/60).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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